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Development of a device to inject a stem cell product into a non-union

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Abstract: *During the development of a device for the injection of stem cells into a non-union, several components had to be considered. Focus was on handling, whereby the radiation exposure for the surgeon and the surgical personnel is to be reduced and the insertion of a depth limiter ensures proper injection of the stem cells into the non-union. Another part of major importance of the device is the cannula. Until now it is not clear if the injection process of stem cells through a cannula influences the vitality of the cells. To investigate the vitality after injection, a test setup was developed in which a cell suspension can be injected with a constant volume flow.*

Introduction

With a world population of 7.6 billion, every year 30.4 million people suffer from a fracture of long bone [1] [2]. A non-union occurs in 5% - 20% of these cases [3]. A non-union is defined either as a fracture without consolidation after 9 months or if clinically or radiologically no further bone healing is expected [4]. Non-unions are subdivided in two groups: hypertrophic and atrophic non-unions. Hypertrophic non-unions are caused by instabilities in the fracture gap, caused for example by inadequate fixation of the fracture. Movement between the fracture ends prevents the formation of a callus bridge, thus preventing union of the bone. The raised contour of the fracture ends can be seen on X-ray images (Figure 1a). The treatment of this hypertrophic non-unions is the mechanical stabilization of the fracture ends by osteosynthesis (Figure 1b).

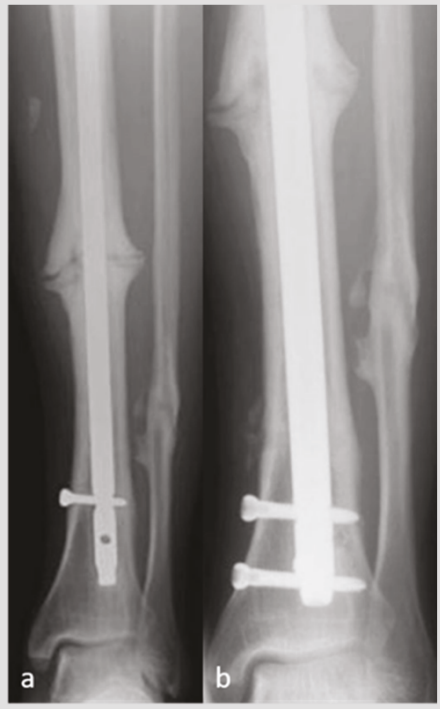

Figure 1: *Hypertrophic non-union in a 29 year old patient, 2 years after nailing osteosynthesis with instability criteria (a) and consolidation sign 4 months after revision (b) [5]*

In atrophic non-unions the viability is disturbed, for example, by a disturbance of the fragment blood flow and vascularization [6]. The loss of viability can have different causes, as initial grade of bone loss, soft-tissue injury or patient dependent factors such as smoking, diabetes or other systemic diseases [7]. The treatment of this type of non-union is considerably more complex than the treatment of a hypertrophic non-union, because there is the need to initiate the bone fracture healing [6]. The classical procedure in the treatment of these types of non-union is the decortication with subsequent autologous bone grafting, in which bone tissue is surgically placed in the fracture gap. The necessary bone tissue is harvested in most cases from the anterior or posterior iliac crest [8]. The additional intervention on the iliac crest is associated with pain and other possible risks for the patient. Therefore, a less invasive treatment of atrophic non-union is desirable. One field of research is the use of mesenchymal stem cells to initiate the bone healing in non-unions.

Mesenchymal stem cells (MSC) are promising for cell-based therapy strategies due to their ability to self-renew and their high differentiation potential in different tissues like osteogenic, adipogenic and chondrogenic tissues [9]. Due to their ability to differentiate into osteogenic cells, they are particularly interesting for regenerative therapies of bones. Mesenchymal stem cells are found in a wide variety of tissues in

the human organism and can be isolated from various tissues like bone marrow, adipose tissue, dermal tissue, umbilical cord blood and synovial fluid [10]. Currently, bone marrow is the most used source for MSC isolation. The bone marrow is thereby in the most cases obtained by iliac aspiration of healthy donors [11]. A disadvantage of this extraction method is the necessary invasive procedure at the donor´s side, which is associated with risks.

Simultaneously, bone marrow and bone fragments which include MSCs, such as femoral heads or bone marrow from intramedullary nailing procedures, are disposed as waste material during bone-related routine surgeries. Therefore, an innovative process chain starting at the collection of the bone material and the isolation of the MSCs to their storage in a cross-border biobank has to be developed. Furthermore, the development of an injection device capable of implanting the cell product into the non-union is necessary.

Development of the Injection Device

Currently, in most cases trephines are used in clinical trials to inject a stem cell product in a non-union [12]. Originally, trephines were developed for biopsies taken from the bone and therefore have some disadvantages for the injection of stem cells in non-unions. The used trephines are usually too long and inconvenient for the injection in long tubular bones. Consequently, exact positioning, which has to be verified by x-ray imaging, is difficult and the exact injection of the stem cell product into the fracture gap is not guaranteed. For this reason, in this contribution a design for a new device to inject a stem cell product into a non-union is presented.

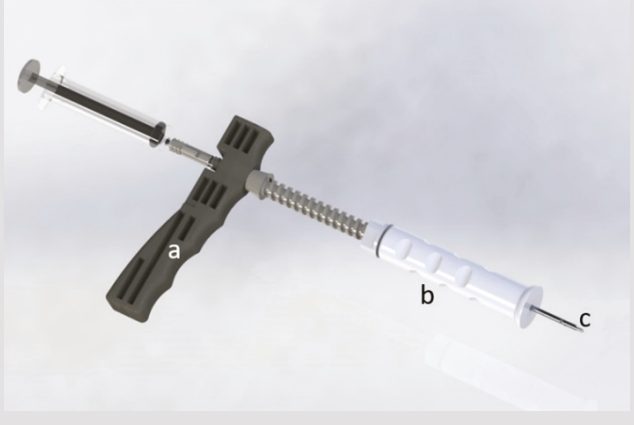

Figure 2: *CAD model of the proposed injection device with ergonomic handle (a), depth limiter (b) and cannula (c).*

Special care has been taken to improve the handling of the device. An ergonomic handle was designed to facilitate the correct positioning in the non-union (Figure 2a). The new handle design ensures optimal control of the applied pressure on the fracture gap. Since the positioning of the device must be checked based on X-ray images, an essential requirement for the device is X-ray compatibility. The device can be held with a single use plastic handle-extender which ensures that the surgeon´s hands are far from the X-ray beam. The injection cannula has to be visible in X-ray imaging and material degradation of the cannula due to the radiation has to be prevented (Figure 2c). Hence, stainless steel is a suitable material for the injection cannula. A further advantage of this material is their approved clinical usage in other surgical instruments, which ensures the biocompatibility. Another important aspect in the selection of useable materials is whether the product is a single-use device or can be reused several times. If it shall be reusable for several times, the material must be sterilizable.

Due to the X-ray based positioning of the device, the surgeon and OR personnel is exposed to ionizing radiation. With currently used injection devices the surgeon must stabilize the device during irradiation to maintain the position, which places the surgeon directly in the X-ray field. For this reason, the handle has been designed in an extendable way. This allows the surgeon to extend the handle during irradiation and thus to hold the device without exposing him directly to the X-ray field.

In addition to the handle, the injection side has been modified. With the devices currently in use, the surgeon is unable to see how far the cannula has already penetrated the bone. The surgeon can find the optimal place for the injection of the cannula only based on several X-ray images, leading to even more exposure to ionizing radiation. To minimize irradiation during placement, a depth limiter was designed above the cannula to allow the surgeon to decide how far the cannula should penetrate the bone (Figure 2b). Accordingly, an X-ray must only be taken before the device is placed to determine the best position and before the injection to check the fit. The depth is adjusted by shifting the depth limiter via a thread. During placement of the cannula in the non-union, pressure is applied to the skin of the patient by the depth limiter. In order to avoid further injuries of the skin at the injection side the surface of the depth limiter was designed to exhibit no sharp edges. Furthermore, care must be taken to ensure the used material for the depth limiter does not lead to irritation of the skin during contact.

A further problem of the currently used trephines depends on the injection side. In the most cases, the cannulas have only one opening. This leads to a unilateral injection direction of the stem cell product in the non-union. For this reason the surgeon cannot precisely control the injection direction of the stem cells in the non-union and an equal distribution of the stem cells is not possible. The next step will be

to develop a cannula with multiple openings on the side, which the surgeon can open and close selectively to accurately determine the direction of injection for optimal results. To avoid negative influence to the viability of the stem cells during the injection process, the occurred forces at the openings must not lead to cell death.

Test setup to simulate the injection process

In order to determine the viability of the stem cells after injection, during the development process of the cannula, a test rig was developed to simulate the injection of stem cell solutions into a non-union, which simulates the shear forces during the injection process in the cannula. The occurring shear forces can possibly lead to tearing of the cell membrane and thus to necrosis of the stem cells. Simultaneously, this test setup will be used to test cannulas which are currently in use, as it has not yet been clarified whether the stem cells are vital after injection through the cannula. If a large number of the injected stem cells are necrotized after the injection, the question must be answered whether the stem cells are directly involved in the healing process or if growth factors, which are released when the cells die, effect the bone healing.

The shear forces are caused by the liquid flow within the cannula and generate mechanical stress on the cells, which can lead to tearing. The strength of the shear forces is significantly influenced by the volume flow. In order to obtain an initial guess order for the magnitude of the volume flow during the injection of stem cells into a non-union, the injection pressure and the volume flow were determined with the help of a surgeon who performed such an injection as part of a clinical study.

To determine the volume flow, the calculation of the counterpressure generated by a non-union during the injection of stem cells was necessary. For this purpose, a capillary was used, which acts as a flow resistor and creates an opposing pressure, which reduces with decreasing length. To approximately produce the same counterpressure as a non-union, the capillary was cut until the surgeon perceived the counterpressure as identical to a non-union. The resulting length of the capillary was 2.47 m with an inside diameter of 0.5 mm. Afterwards the volume flow was measured by injection of 0.4 ml fluid through the capillary by the surgeon and the pressure was increased over time. Since the number of cells in the solution for injection is very low and has no influence on viscosity, water was used for the experiments with a viscosity of 0.001 Pas.

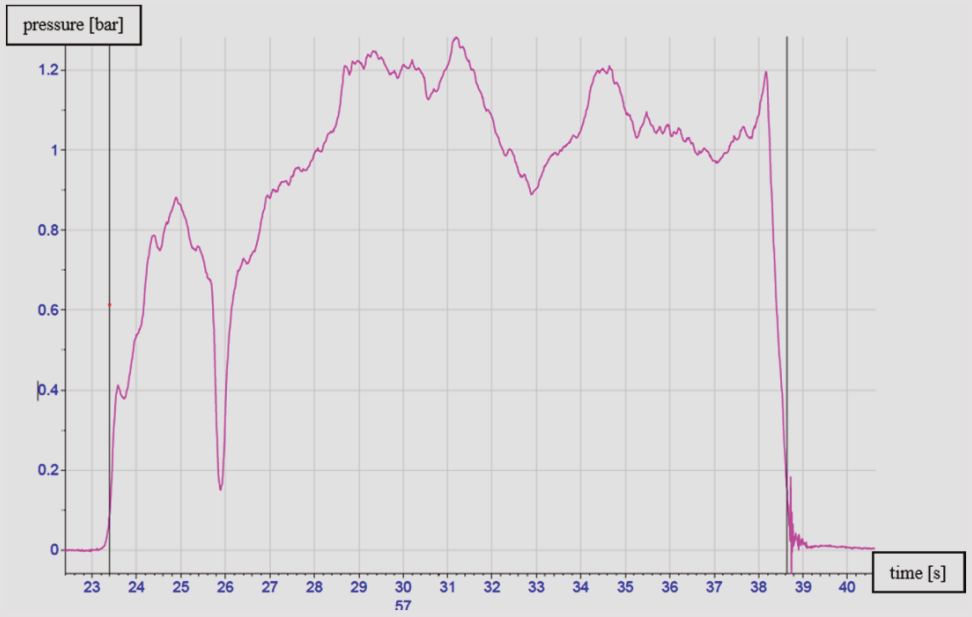

Figure 3: *Pressure curve to determine the volume flow. Due the pressure changes cause by the manual injection an averaged pressure value has to be used.*

The average volume flow $\dot{V}$ was calculated using the following formula:

$$\dot{V} = \frac{\pi \cdot r^4 \cdot \Delta p}{8 \cdot \eta \cdot l} . \quad (1)$$

With r the radius of the capillary, Δp the mean pressure in the capillary, η the viscosity of water and l the length of the capillary. Figure 3 shows a typical pressure curve for the volume flow estimation. Due to the pressure fluctuations caused by the manual injection an averaged pressure is used.

Table 1: *Calculated mean volume flow according to equation (1).*

Measurement	Average pressure [bar]	Average volume flow [ml/min]
1	1.0074	16.85
2	0.9677	16.19
3	0.7162	11.98
4	1.1855	19.83
5	0.776	12.98
6	0.9615	16.08
Mean	0.9357	15.65
Standard deviation	0.17	2.83

The results shown in table 1 indicate the volume flow is about 15.65 ml/min and the mean pressure 0.9357 bar. Both measurements show small variations due to the fact the injections were carried out manually and the pressure could not be kept constant during the injection. However, these fluctuations are not further important as only an order of magnitude of the volume flow should be determined.

For the development of the test setup, the second step was to determine which pressure resistances influence the total pressure. The total pressure is made up of two parts: the pressure drop in the cannula, which represents a flow resistance, and the pressure drop in the tissue of the non-union, which has a solid tissue matrix generating a counterpressure during injection. To identify the total pressure, it is sufficient to determine one of the two parameters and draw conclusions about the other one. The pressure drop of the cannula was estimated with a constant volume flow of 15.65 ml/min. Figure 4 shows a typical pressure curve for the pressure drop in the cannula. Since the measurement is quite noisy the average pressure drop is used and the mean value over different measurements has been calculated along with the respective standard deviation (Table 2).

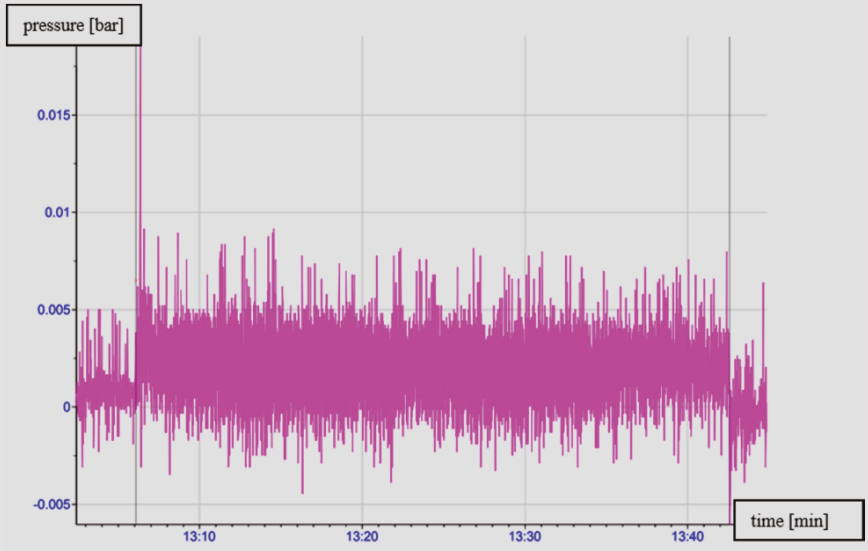

Figure 4: *Pressure drop over the cannula. Due to the noisy measurement the use of an averaged pressured drop is necessary.*

Table 2: *Mean value and standard deviation of the averaged pressure drops in the cannula.*

Measurement	Average pressure [bar]
1	0.0038
2	0.0023
3	0.0022
4	0.0032
5	0.0049
6	0.0037
Mean	0.00335
Standard derivation	0.00093

The pressure drop over the cannula is very low with a very small standard deviation. These deviations can be explained to possible measurements inaccuracies of the pressure sensor. The pressure drop is 0.00335 bar, which corresponds to 0.36% of the total pressure exposure during the injection into a non-union. Therefore, the pressure drop in the cannula can be neglected. The counterpressure is mainly generated in the tissue of the non-union.

Various components are required for the construction of the test setup (Figure 5). The syringe holder is one of the components, which was designed such that the use different syringe sizes (1, 5 and 10 ml) is possible. Another important point for the developed setup is the injection of the stem cells with a constant volume flow. To guarantee a constant volume flow the injection should be carried out automatically. Therefore, a linear actuator, which can move a carriage, by means of a threaded rod, was used to move the plunger of the syringe. To enable the movement, the threaded rod runs in a threaded nut inserted in the slide. This allows the carriage to move forwards and backwards depending on the direction of rotation, enabling the plunger to move forwards and backwards. The linear actuator is controlled by a stepper motor, which can adjust the volume flow in the range of $10 - 30$ ml/min. To comfortably perform the measurements a user interface has been designed to run on a personal computer.

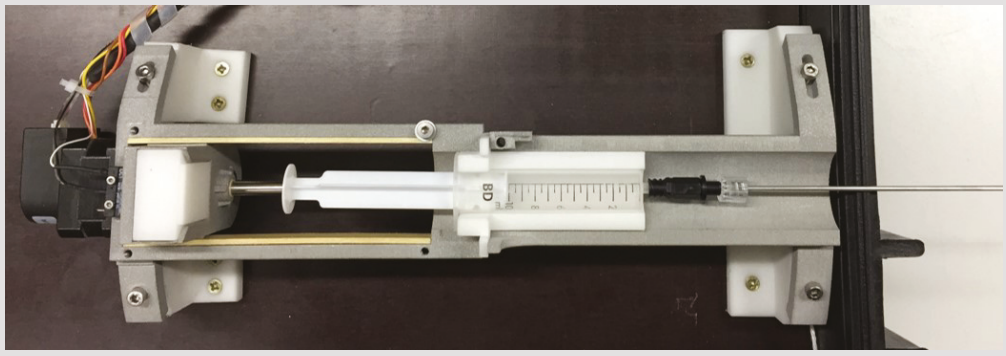

Figure 5: *Construction of the test up to investigate the influence of the volume flow on the viability of the stem cells.*

After finalisation of the test setup, a control measurement was carried out to validate the volume flow. Measurements were taken with each syringe size (1, 5 and 10 ml) with two different volume flows (20 and 30 ml/min) and with different injection volumes, dependent on the syringe sizes. The volume flow was validated by comparing the measured injection time with a calculated time, which can be calculated from the definition of the volume flow according to the following formula:

$$\dot{V} = \frac{dV}{dt} \Rightarrow t = \frac{V}{\dot{V}}. \tag{2}$$

Table 3: *Comparison of calculated time and measured time for volume flow validation.*

Syringe size [ml]	Volume flow [ml/min]	Injection volume [ml]	Calculated time [s]	Measured time [s]	Difference [s]
10	20	10	30.00	30.40	0.40
		5	15.00	15.31	0.31
	30	10	20.00	20.04	0.04
		5	10.00	10.28	0.28
5	20	5	15.00	15.12	0.12
		3	9.00	8.90	0.10
	30	5	10.00	10.00	0
		3	6.00	6.00	0
1	20	1	3.00	2.80	0.20
		0.5	1.5	1.47	0.03
	30	1	2.00	2.26	0.26
		0.5	1.00	0.89	0.11

The results of the measurements shown in Table 3 indicate a constant volume flow is possible with the developed setup. The maximum difference of 0.4 s between the

calculated and measured time can be explained by the manual measurement of the time using a stop watch.

Conclusions

For the application of mesenchymal stem cells in treatment of atrophic non-unions, no proper injection tool is available up to now. The use of trephines is widely spread but suffers from severe disadvantages. The handle of a trephine is hardly useable for delicate application, its short length forces the surgeon to operate directly in the main field of X-ray radiation while placing the needle properly, leading to unnecessarily high exposure to radiation. Moreover, no proper injection depth control is available. The device presented in this contribution solves these disadvantages by an ergonomically designed handle, which allows precise control of the device, the extended length of the device enables the surgeon to place the needle under x-ray-based evaluation without exposure to the main radiation field and the depth limiter ensures an exact injection depth.

Injection of mesenchymal stem cells into a non-union bears the risk of cell destruction due to high forces leading to tearing of the cell membranes. The destructive forces depend on the applied volume flow. Therefore, a setup has been developed to investigate the influence of the volume flow on the viability of the stem cells. The setup ensures a constant volume flow into a tubular capillary, which has a flow resistance comparable to a non-union, thus leading to comparable forces acting on cell membranes in a test sample. The setup has been validated in terms of influence of the syringe cannula on the overall pressure in the system, as well as the capability of producing a constant flow volume. The results indicate a neglectable influence of the syringe cannula and a feasible control of the volume flow.

Further experiments with the presented injection device and the test setup for cell viability aim for a complete and reliable process description for injection of mesenchymal stem cells into a non-union.

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